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Proinflammatory Cytokines in Heart Disease

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Abstract
Proinflammatory cytokines affect nearly all tissues and organ systems, and the vasculature is no exception. Although a considerable amount of research has focused on the role of the two most prominent proinflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF), in the pathogenesis of sepsis and septic shock, the role of these and other cytokines in the pathogenesis of atherosclerotic lesions of the coronary artery, the acute ischemic event associated with myocardial infarction, the progression of myocardiopathies or the loss of myocardial function in congestive heart failure is a relatively recent discovery. Moreover, there has also been significant investigation of the cardioprotective effects of cytokines. Most of the attention has focused on the acute coronary syndromes and the myocardial suppression that takes place as a result of acute ischemia.

The potential for anticytokine-based therapies in treating heart disease is great. Parenteral TNF-α neutralization and IL-1 receptor blockade are presently used to treat rheumatoid arthritis. Two orally effective agents, the IL-1β-converting enzyme inhibitor and the mitogen-activating protein kinase p38 inhibitor, are currently being investigated in clinical trials.

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General Considerations of Cytokine Biology as It Relates to Heart Disease

It must be emphasized that measuring cytokines in human disease and making direct correlations between any particular cytokine and any particular disease is not proof that the cytokine plays a causal role in the disease process. This is also the case with assessing the role of cytokines in the pathogenesis of atherosclerotic lesions of the coronary artery, the acute ischemic event associated with myocardial infarction, the progression of mycardiopathies or the loss of myocardial function in congestive heart failure. Nevertheless, a highly significant correlation of the level of a particular cytokine or cytokines with the severity of disease contributes to the understanding of the

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role of cytokines in that disease process. There are two experimental approaches that do provide absolute evidence of a causal role of any particular cytokine in the pathogenesis of a particular disease. First, in animal models of the disease, if administration of highly specific cytokine antagonists reduces the severity of the disease. In heart disease, several animal models exist which have application to human disease. Animal models include gene deletion studies or transgenic mice overexpressing specific genes. Second, specific blockade or neutralization of some cytokines, namely interleukin-1 (IL-1) and tumor necrosis factor (TNF), is now possible in patients, as these agents are currently used in humans with rheumatoid arthritis with a fairly low incidence of side effects or problems. In fact, the role of TNF in myocardial dysfunction of chronic heart failure is now being directly assessed in patients given soluble TNF receptor (p75) fused to the Fc domain of IgG (p75-Fc, or Enbrel). A preliminary study suggested that Enbrel administered to patients with moderately severe heart failure exhibited increased exercise tolerance.

Direct Effect of IL-1 and TNF on Myocardial Function

In studies on the effects of cytokines on myocardial function in septic shock, the data have been primarily physiological and derived from experimental models of septic shock. In those animal models, intravenous infusion of IL-1 or TNF, but particularly the combination of IL-1 plus TNF, exhibited a profound ability to suppress myocardial function. There is little doubt that the amount of IL-1 or TNF that is employed to show myocardial suppression in these experimental animal models is rather high, and some investigators question whether these concentrations are clinically relevant. However, following intravenous infusion of recombinant cytokines such as IL-1 or TNF into rodents or rabbits, there is rapid excretion and tissue levels are low in comparison to blood levels. On the other hand, local production of cytokines from the myocardium itself (as would take place in ischemia) is more likely to affect myocardial function at concentrations that are clinically relevant. For example, in human atrial muscle strips suspended in an oxygenated balanced salt buffer and electrically paced, contractile function as measured by developed force is dramatically reduced by the presence of less than 10 pg/ml of exogenous TNF-α in the bath [1]. IL-1β induces a similar reduction in developed force at similarly low concentrations. Therefore, the

human heart appears to be exquisitely sensitive to the direct effects of IL-1 and TNF, and in both cases, these cytokines result in depression of function.

The first evidence that IL-1 and TNF directly suppress myocardial function came from studies on human serum of patients with acute septic shock marked by reversible myocardial depression [2]. Using immunoabsorption, removal of both TNF-α and IL-1β (but not either alone) from these sera resulted in the elimination of serum myocardial depressant activity. IL-2, IL-4, IL-6, IL-8, IL-10 and interferon (IFN)-γ failed to cause significant cardiac myocyte depression over a wide range of concentrations. Individually, TNF-α and IL-1β each resulted in depression of peak velocity and myocyte shortening in vitro, but the combination of TNF-α plus IL-1β induced depression of myocardial cell contractility at substantially lower concentrations, consistent with a synergistic effect [2]. The role of nitric oxide (NO) and cyclic guanyl monophosphate in the depression of myocardial contractility induced by either IL-1β, TNF-α or the combination of these cytokines was investigated [3]. Inhibitors of NO synthase such as N-methyl-L-arginine (L-NMA) and methylene blue for the inhibition of guanylate cyclase prevented cytokine-induced myocardial suppression; an excess of L-arginine with L-NMA restored the effect. In addition, TNF-α, IL-1β or TNF-α plus IL-1β induced NO formation in myocyte cultures; moreover, sera from patients with acute septic shock also induced NO production.

In considering the direct effect of IL-1 or TNF (or other cytokines) on myocardial function, one should keep in mind several pathogenic situations. These are: (1) the local production of IL-1 and TNF from resident myocardial macrophages; (2) the ability of ischemia to induce IL-1 and TNF from these macrophages; (3) the level of IL-1 and TNF receptor expression on myocytes, and (4) relevant postreceptor events following IL-1 and TNF triggering of their respective receptors on myocytes.

IL-1 and TNF Production during Acute Ischemia

TNF-α and IFN-γ production by peripheral blood mononuclear cells was assessed in 8 control subjects, 10 patients with stable angina pectoris and 10 patients with unstable angina pectoris. Secretion of both TNF-α and IFN-γ by peripheral blood mononuclear cells increased progressively over 48 h, and it was consistently higher (p < 0.02) in patients compared with control subjects. A similar increase in cytokine secretion was observed in patients with stable or unstable angina pectoris. However, there was no relation between the severity of coronary artery disease by angiography and cytokine secretion [4]. In

another study of patients with heart failure due to dilated cardiomyopathy, TNF-α concentrations were elevated only in heart failure patients with coronary artery disease, particularly in patients in acute failure [5]. TNF-α was also elevated in patients with coronary artery disease and chronic heart failure compared to coronary artery disease patients without failure.

Measurement of IL-1β levels in heart disease is more difficult because the secretory pathways of this cytokine are more complex than for those of TNF-α. Instead of measuring IL-1β, it is more reliable to measure IL-1 receptor antagonist (IL-1Ra), which has a secretory peptide and is a member of the IL-1 family. In fact, IL-1Ra measurements correlated with acute-phase proteins, IL-6 levels and IL-1β levels in several studies. IL-1Ra is an acute-phase protein [6]. Forty-three patients admitted to a coronary care unit for Braunwald class IIIB unstable angina were studied for serum levels of IL-1Ra and IL-6. IL-6 levels often reflect the levels of biologically active IL-1β plus TNF-α. Patients were treated identically with anticoagulants and aspirin. One group of patients had an uneventful course, whereas a second group had a complicated in hospital course. The group of uncomplicated patients revealed decreased IL-1Ra and IL-6 levels at 48 h, but IL-1Ra and IL-6 levels increased by 37 and 57%, respectively, at 48 h in the group with a complicated hospital course (p < 0.01) [7].

Blockade of TNF during Acute Ischemia

In isolated, perfused rat hearts, adenosine decreased ischemia-induced cardiac TNF-α production and improved postischemic functional recovery [8]. This study demonstrated that ischemia alone induces an increase in cardiac tissue TNF-α in a crystalloid-perfused model and that adenosine decreases cardiac TNF-α and improves postischemic myocardial function. We have also used a model of perifused human atrial tissue taken at the time of cardiopulmonary bypass surgery to assess the role of TNF in global ischemia-reperfusion. When the tissue is exposed to an ischemic insult lasting 30 min, recovery of developed (contractile) force paced at 1 Hz is 20–40% when reexposed to oxygenation. Human atrial trabeculae were obtained during cardiac surgery and suspended in organ baths, paced at 1 Hz, and developed force was recorded [9]. Ischemia-reperfusion decreased human myocardial developed force to 18% of baseline. Pretreatment with adenosine decreased ischemia-induced myocardial TNF-α production and increased postischemic contractile force to 39%. Specific blockade of TNF using TNF binding protein, the extracellular domains of the

TNF receptor p55, also improved the postischemic function of human myocardium (55% of baseline) [9]. To date, specific blockade of IL-1 with IL-1Ra has not been tested in this model. However, given the marked synergism of TNF-α plus IL-1β in depressing cardiac muscle strength [1], it is likely that IL-1 blockade will also reduce ischemia-induced myocardial suppression. In a liver ischemia model, pretreatment with IL-1Ra reduced tissue injury [10].

IL-1β suppresses the spontaneous rhythmic beating of cultured neonatal rat myocytes, an effect inhibited by the addition of transforming growth factor-β [11]. Exposure of excised papillary muscle to IL-1 in vitro prolonged the duration of the action potential and refractory period, and both effects were reversed by cyclooxygenase inhibitors [12]. Similar suppressive effects of IL-1 have been reported using isolated myocytes stimulated with β-adrenergic agonists [13]. Because IL-1 induces NO synthase, some effects of IL-1 on isolated hearts may be mediated by NO. Although no specific IL-1 receptor blocking studies have been reported, IL-1 produced during myocarditis may affect myocardial function. For example, IL-1β mRNA was found in endomyocardial biopsies from patients with myocarditis, and elevated IL-1RI gene expression was detected in samples from dilated cardiomyopathies [14]. Treating mice with IL-1 or TNF induced autoimmune myocarditis in mice infected with Coxsackie B3 [15].

Cytokines and the Development of Atherosclerotic Vascular Disease

There is another important consideration in the pathogenesis of coronary artery disease relevant to proinflammatory cytokines: the role of these cytokines in the development of the atherosclerotic plaque. This area of research is more complicated to define than is the role of cytokines in acute myocardial suppression associated with an acute ischemic event. However, there is no question that in the chronic inflammatory process that is part of plaque formation, there is a clear role of IL-1 and TNF. As discussed below, there is now direct evidence that IL-1 is important to this process.

Similar to cultured fibroblasts, cultured human endothelial and smooth muscle cells derived from healthy blood vessels produce IL-1 when stimulated by a variety of agents, including endotoxin, TNF or IL-1 itself [16–19]. Primary endothelial cells grown on an inert surface release IL-1 when subjected to laminar flow-induced
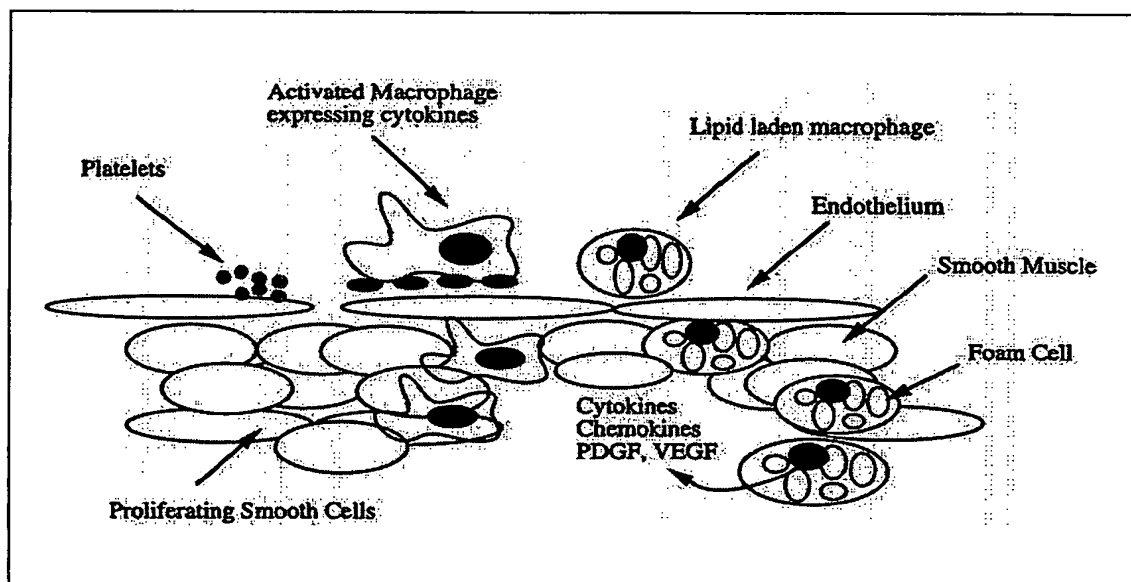
Fig. 1. Proposed mechanisms for proinflammatory cytokine contribution to the formation of atherosclerotic lesions. The endothelial layer encounters three cytokine-related events. As shown on the left side, platelet contact with the endothelial surface results in the release of cytokines such as IL-1β. IL-1β, in turn, activates the endothelial surface to increase adhesion molecule expression. Activated macrophages expressing cytokines adhere to the activated endothelium and release cytokines. In addition, activated macrophages emigrate through the endothelial layer where they continue to produce cytokines. Lipid-laden circulating macrophages also adhere to the endothelial surface and penetrate into the subendothelial space, where they are recognized as foam cells. Foam cells and macrophages release cytokines, chemokines and growth factors in the subendothelial lesion. The respective effects of proinflammatory cytokines, chemokines and growth factors contribute to the progression of the atherosclerotic lesion. PDGF = Platelet-derived growth factor; VEGF = vascular endothelial growth factor.

shear stress (6 dyne·cm^2) [20]. Using in situ hybridization, IL-1α and IL-1β mRNA have been detected in endothelial cells of various organs taken from apparently healthy animals. However, the function of constitutive IL-1β expression in endothelial cells is unclear, since mice deficient in IL-1β have normal tissue phenotypes [21, 22]. Using specific cDNA probes for rabbit IL-1α and IL-1β [23], freshly homogenized or cultured aortic tissue did not show spontaneous expression of either IL-1 gene [24].

Thoracic and abdominal aortic segments taken from hypercholesterolemic monkeys spontaneously express the gene for IL-1β. This expression was associated with an increase in platelet-derived growth factor B chain synthesis in lipid-filled macrophages (foam cells) that had invaded the atheromatous lesions [25]. Using immunohistochemical staining, each of 55 sclerotic vein coronary artery bypass grafts revealed the presence of IL-1α, whereas nonoccluded internal mammary artery grafts did not

[26]. The affected vessels also showed myointimal proliferation, reduced luminal patency and macrophage infiltration. Cultured tissue minces from human abdominal aneurysms release significantly more IL-1β compared to control aortic tissue from cadavers [27]. The presence of IL-1 in atheromatous tissues is thought to be due to infiltrating foam cells which synthesize IL-1 in response to oxidized low-density lipoproteins (LDL). Experimental data have shown that LDL of varying oxidation states and associated linoleate oxidation products stimulate normal human blood monocytes to produce IL-1β [28, 29]. Reducing lipoprotein peroxidation by antioxidants or adding antioxidants to lipopolysaccharide-stimulated IL-1 production reduced IL-1 synthesis [30].

IL-1 can also act as a growth factor for smooth muscle cell proliferation in the absence of inhibitory prostaglandins [31]. Early intimal thickening of coronary arteries in transplanted pig cardiac allografts was associated with IL-1-induced fibronectin deposition [32]. It is unlikely that

IL-1 is acting directly as a growth factor in proliferative vascular lesions but rather increases the expression of traditional vascular cell mitogens such as platelet-derived growth factor and fibroblast growth factor and their respective receptors (fig. 1). Furthermore, IL-1 often acts synergistically with these vascular mitogens. Long-term blockade of IL-1 receptors or decreasing IL-1 synthesis or release in the vascular lesion would help identify an essential role for IL-1 in the pathogenesis of vascular lesions. Slow local release of IL-1Ra from a polymer sleeve surrounding a damaged sciatic nerve in the mouse reduced neovascularization of the damaged area [33]. Angiotensin-converting enzyme inhibitors appear to reduce the restenosis of grafted vessels, and in vitro, these inhibitors reduce gene expression and synthesis of IL-1 and TNF [34].

Ischemia-reperfusion in the liver, brain, lung and myocardium results in the synthesis of IL-1, TNF and other cytokines. Because of their importance to the infiltration of inflammatory cells, IL-8 and other chemokines released after ischemia-reperfusion contribute to local tissue damage. Moreover, the production of IL-8 is often under the control of IL-1 [35]. In the case of ischemic injury due to coronary occlusion, myocardial muscle damage correlates with neutrophilic infiltration. In dogs, acute coronary occlusion results in the accumulation of myocardial neutrophils and infarction; however, IL-1 receptor blockade with IL-1Ra reduced neutrophilic infiltration and infarct size by 50%.

Role of Macrophage Membrane and Platelet IL-1

Nearly all working hypotheses of the pathogenesis of atherosclerotic vascular disease implicate a role for the lipid-filled invading macrophage and the activated platelet. Several laboratories have demonstrated biologically active membrane-bound IL-1α [36, 37], and immunohistochemical staining in diseased blood vessels also reveals the presence of IL-1α [26]. Human monocytes which have been fixed after lipopolysaccharide activation express IL-1α on their surface, and when incubated with cultured human endothelial cells, induced IL-8 production [38]. Moreover, nearly all the IL-8-inducing activity was blocked by either an antibody to IL-1α or IL-1Ra, suggesting that no other membrane cytokine, for example TNF, was involved.

In a similar fashion, epinephrine-activated human platelets induce IL-8 synthesis from endothelial cells and

nearly all this activity was blocked by IL-1Ra [39]. However, the IL-1 biological activity of activated platelets appears to be due to IL-1b rather than IL-1α [40, 41], and IL-1 activity was associated with platelet membranes rather than cytosolic extracts [39]. Thrombin-activated platelets induce ICAM-1 and ELAM-1 expression as well as synthesis of GM-CSF and IL-6 from cultured endothelial cells, and these activities are neutralized by an antibody to IL-1β, not IL-1α [41]. The increased expression of adhesion molecules by either soluble or platelet-associated IL-1 likely contributes to the role of the platelet in the inflammatory phase of the atherosclerotic lesion. A study has demonstrated IL-1 receptors on approximately 5% of circulating platelets from healthy donors, but on 10% from patients with inflammatory bowel disease [42]. In addition, IL-1 activates normal platelets, as assessed by a dose-dependent increase in the expression of the leukocyte adhesion molecule p-selectin (CD62) and the fibrinogen receptor [42].

Taken together, IL-1β activity on activated platelets appears to be a consistent finding. Disruption of the endothelial surface is not completely understood, but the subintimal location of activated monocytes and platelets expressing surface IL-1 activity likely contribute to the early events of the atherosclerotic lesion. Hyperlipidemia and oxidized LDL activate IL-1 production by macrophages, and this event may be part of the initiation process. Once a pathological process is associated with the presence of IL-1-producing cells or IL-1 activity on cell membranes, the multiple biological effects of IL-1 as an inflammatory mediator implicate this cytokine. However, there are no long-term studies in atherosclerotic-prone hypercholesterolemic animals in which specific blockade of IL-1 receptors has been examined, and hence the 'IL-1 component' of the atherosclerotic lesion remains unclear. Only short-term postischemic changes have been studied and revealed a significant reduction by IL-1Ra. Blocking TNF will also reduce ischemic damage. Although it is not surprising to implicate an activated macrophage in an IL-1-mediated process, the unusual finding of IL-1 activity on platelets is particularly important for vascular diseases. How does IL-1β become associated with platelets? Uptake of plasma proteins by platelets has been shown [43], and either free IL-1β or IL-1β bound to the soluble receptor type II could explain this finding.

Role of IL-1 in the Pathogenesis of Arteritis

In two separate publications this year, researchers reported that knockout mice which cannot produce their own IL-1Ra spontaneously develop inflammatory and autoimmune-like diseases [44, 45]. IL-1Ra is a naturally occurring protein structurally very similar to IL-1 itself. IL-1Ra functions as a pure receptor antagonist, preventing a biological response only to IL-1 [46]. When IL-1 occupies its receptor, various proinflammatory events are initiated; when IL-1Ra occupies the IL-1 receptor, however, there is no response. Furthermore, when receptors are occupied by IL-1Ra, IL-1 itself cannot bind to cells. Receptors for IL-1 are found on cells with various biological functions, including cells mediating inflammation and tissue remodeling. In addition, IL-1 induces the synthesis of chemokines, which facilitate the emigration of neutrophils, macrophages and lymphocytes into tissues. Therefore, it was of considerable importance that mice lacking the ability to produce their own IL-1Ra were susceptible to developing spontaneous disease. The implication of these mouse studies is that there is a balance in the host between the proinflammatory activities of IL-1 and the ability of IL-1Ra to keep those activities in check by occupying IL-1 receptors.

The IL-1Ra knockout mice spontaneously develop a lethal arterial inflammation characterized by transmural infiltration of neutrophils, macrophages and T lymphocytes [45]. The lesions were commonly observed at the areas of high vessel turbulence, which in humans are also areas of atherosclerotic plaque formation. The arteritis in these mice resulted in stenosis with infarction of organs as well as hemorrhage from aneurysmal rupture which resulted in death. The inflammatory nature of the lesions is consistent with the biological activities of IL-1 on endothelial cells and smooth muscle cells. Heterozygotes, that is the offspring of one parent mouse producing normal amounts of IL-1Ra with a parent mouse not producing IL-1Ra, also developed arterial lesions but with less inflammation.

The IL-1Ra knockout mice lived in clean but not germ-free animal facilities and were free of obvious or detectable infections. The littermate control mice, producing their own endogenous levels of IL-1Ra, did not develop arteritis. One can conclude from these knockout studies that the levels of IL-1Ra produced in the control mice are sufficient to protect the animals from the biological consequences of IL-1 produced under natural living conditions. The studies add to existing data that IL-1 produced during infection, inflammatory or autoimmune disease con-

tributes to the pathological process. It appears that if an individual produces sufficient IL-1Ra to occupy a sufficient number of IL-1 receptors, then IL-1-mediated inflammation and tissue destruction is held at bay. On the other hand, if the amount of IL-1Ra endogenously produced by an individual during disease is insufficient and unable to control IL-1 activity, runaway inflammation takes place. Hence, agents that specifically block IL-1 or reduce its production will likely contribute to the treatment of inflammatory disease.

Both IL-1 and IL-1Ra are elevated in disease; hence, administration of IL-1Ra is a therapeutic strategy in treating inflammatory and autoimmune conditions [46]. In fact, in double-blind, placebo-controlled trials, administration of IL-1Ra has been shown to significantly reduce inflammation and new joint bone erosions in patients with moderately severe rheumatoid arthritis [47, 48]. Over 2,000 patients have received IL-1Ra for the treatment of rheumatoid arthritis, some for more than 6 years. IL-1Ra is presently being investigated in trials for other autoimmune diseases and is awaiting FDA approval.

Acknowledgment

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